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The overall goal of this project has been to learn about the structure, developmentally regulated synthesis and assembly of a family of secretory proteins (SPs) that are synthesized in salivary glands of an aquatic insect and assembled into an insoluble biopolymer of silk-like threads. Molecular biological and immunological techniques were used to ascertain the structure of two additional SPs and the developmental regulation of the expression of their genes. A 185/220-kDal Cys-rich SP homologue has been identified in three species of Chironomus. Biochemical, biophysical and electron microscopic techniques were used to study SP disassembly/reassembly in vitro and measure the higher order structure of of 1000-kDal SPs and their repeated domains in the form of synthetic peptides. This domain approach to protein structure was extended to insect chorions.

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FINAL REPORT

GRANT#: NOOO14-87-K-0387 R&T CODE: 441c-028

PRINCIPAL INVESTIGATOR: Dr. Steven T. Case

INSTITUTION: The University of Mississippi Medical Center

GRANT TITLE: Secretory Polypeptides Encoded in Balbiani Ring Genes

PERIOD OF PERFORMANCE 15 APR 1987 - 31 JAN 1992

OBJECTIVE: To learn about the structure, developmentally regulated synthesis and assembly of a family of secretory proteins (SPs) that are synthesized in salivary glands of an aquatic insect and assembled into an insoluble biopolymer of silk-like threads.

ACCOMPLISHMENTS (All Years):

[1] Primary structure of SPs/expression of their genes.

(a) We determined that the primary sequence of two additional SPs (sp140 and sp185) consists of repeated motifs. We also studied the developmentally regulated expression of their genes. A third putative SP-coding cDNA was studied in detail, but we were unable to unambiguously identify the protein encoded by the 4.8-kb mRNA. These studies led to extensive use and characterization of multiple antigenic peptides for antibody production.

(b) Antipeptide antibodies were used to search for conserved and diverged epitopes in SPs from different species of Chironomus. The only conserved epitope found was on sp185 from C. tentans and C. pallidivittatus and sp220 in C. thummi. Amino acid analyses, performed by J.H. Waite, indicates that all three proteins are very similar in composition, particularly in their high (about 17%) content of Cys. Oligonucleotides from C. tentans have selected cDNA clones for future analysis from libraries of the other two species.

(c) A cDNA expression library was made to clone the cDNA for a "special secretory protein (ssp160) found in only four cells of Chironomus thummi salivary glands. To screen the library, a polyclonal antibody was raised against gel-purified ssp160. While the specificity of the antibody was demonstrated on Western blots, we were unable to select any clones. Controls have since shown that the ssp160 antibody reacts primarily with N-linked sugars, a post-translational modifcation of proteins that bacteria

fail to make. Future selection of this SP-encoding cDNA clone will require alternate strategies.

[2] Higher order structure and assembly of SPs

(a) We demonstrated that SPs exist in vivo as dissociable supermolecular complexes (fibrils and beaded fibers) that may be intermediates in the pathway for their assembly into insoluble silk. These complexes are stabilized by electrostatic interactions and disulfide bonds. We described the first assay for Chironomus SP disassembly/reassembly in vitro and showed that spIs, the 1000-kDa SPs, are the fibrous backbone of larval silk.

(b) Circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopy were used to ascertain the structure of synthetic peptides that correspond to alternating "subrepeat" (SR) and "constant" (C) domains of tandem core repeats within spIa, one of the 1000-kDal SPs. SR peptide is mainly composed of poly(Gly)II-helix while C peptide is mainly α-helical; both are punctuated with ß-turns. Thus far, we have failed to obtain either crystals or laser Raman spectra of these peptides.

(c) Conditions have been found whereby fully reduced C peptide, which contains four Cys residues, can associate in vitro. One aggregated form is a fully reducible dimer apparently devoid of free sulfhydryls. In contrast, we found a non-reducible form which is the by-product of one of the steps used for purification of C peptide. This latter form is multimeric (dimer through tetramer) and apparently stabilized by a covalent bond(s). The nature of this bond(s) has not been determined. Under no circumstances have either C or SR peptides aggregated into supermolecular complexes resembling those formed by spIs in vitro. These data suggest that the minimum unit for assembly in vitro must be at least a [C+SR] monomer.

(d) During the performance of this contract we learned that synthetic peptides can provide substrates of superior purity for biochemical and biophysical studies of protein domains, especially imperfect tandem repeats. We gained considerable experience in peptide synthesis and analysis by CD and FTIR spectroscopy and comparing such data to that obtained for the cognate protein from which the peptides were derived. We applied this experience to a related biopolymer. In collaboration with Dr. Stavros Hamodrakas at the University of Athens, we used this approach to study the structure of repeated domains in egg-shell chorion from silk moths and lepidopterans. In contrast to Chironomus SPs, the structure of these peptides and proteins is mainly ß-sheet.

SIGNIFICANCE:

[1] Identification of additional SPs brings us closer to describing all members of this novel protein family that can assemble into insoluble silk. Their developmentally regulated synthesis suggests that while larval and prepupal silks look similar, their composition and, perhaps, physical properties are different. Sequence comparisons of repeats within SPs and homologous proteins in related species help to pinpoint conserved sites that may be crucial for intraprotein and protein-protein interactions. It's becoming apparent that SPs represent a completely novel class of structural proteins.

[2] The structural studies of C and SR peptides suggest that spIs have a unique structure: they are 1000-kDa fibrous proteins comprised of alternating domains of α-helix and poly(Gly)II helix. This structure would result in a biopolymer with a unique alternation of contrasting physical properties such as flexibility, extensibility, elasticity and strength. The inability of C and SR peptides to form supermolecular aggregates suggests that neither contain the minimum structural information required for assembly of fibers. However, under certain conditions, C peptide does have the intrinsic property to form reducible and non-reducible multimeric units. This may prove useful for designing a hybrid self-assemblying biomaterial.

PUBLICATIONS (All Years):

1. Dignam, S.D., Yang, L., Lezzi, M. and Case, S.T. Identification of a developmentally regulated gene for a 140-kDa secretory protein in salivary glands of Chironomus tentans larvae. J. Biol. Chem. 264:9444-9452 (1989).

2. Wellman, S.E. and Case, S.T. Disassembly and reassembly in vitro of complexes of secretory proteins from Chironomus tentans salivary glands. J. Biol. Chem. 264:10878-10883 (1989).

3. Dignam, S.S. and Case, S.T. Balbiani ring 3 in Chironomus tentans encodes a a 185-kDal secretory protein which is expressed throughout the fourth larval instar. Gene 88:133-140 (1990).

4. Case, S.T., Wellman, S.E. and Hamodrakas, S. Assembly characteristics and structural motifs in an aquatic insect's biopolymer. Mater. Res. Soc. Symp. Proc. 218:233-237 (1991).

5. Case, S.T. and Wieslander, L. Secretory proteins of Chironomus salivary glands: structural motifs and assembly characteristics of a novel biopolymer. Res. Prob. Cell Diff. (in press, 1992).

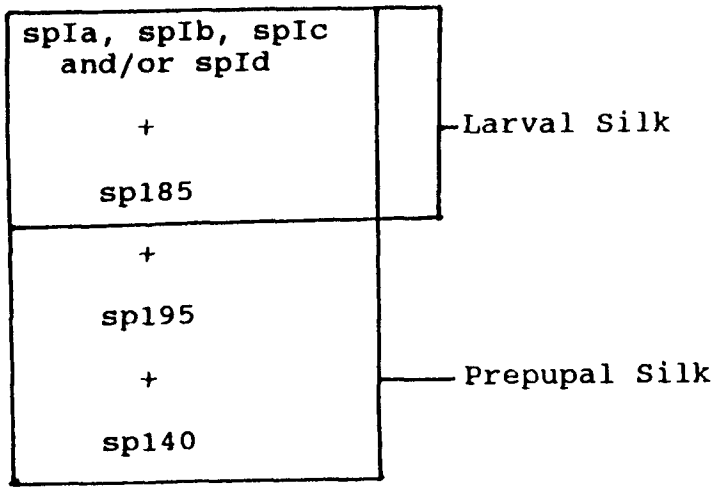
6. Wellman, S.E., Hamodrakas, S.J., Kamitosis, E., and Case, S.T. Secondary structure of synthetic peptides derived from the repeating unit of a giant secretory protein from Chironomus tentans. (submitted, revision under review).

7. Brumley, L.L., Hong, A.L. and Case, S.T. Immunochemical identification of uncharacterized proteins using multiple antigenic peptides for antibody production. (manuscript in prepartion).

8. Brumley, L.L., Bogachev, S.S., Kolesnikov, N.N. and Case, S.T. Conserved and diverged epitopes among secretory proteins from Chironomus salivary glands. (manuscript in preparation).

9. Hamodrakas, S.J., Ottensmeyer, F.P., Wellman, S.E. and Case, S.T. Solution structure and assembly of silkmoth chorion proteins. (manuscript in preparation).

10. Hamodrakas, S.J., Orfanidou, C. Ottensmeyer, F.P., Wellman, S.E. and Case, S.T. Structural studies of the in vitro reconstitution of chorion proteins from the lepidopterans, Manduca sexta and Sesamia nonagrioides. (manuscript in preparation).



OBJECTIVES

Assembly of Secretory Proteins (SPs) into Insoluble Biopolymers

- Learn primary structure of SPs by cloning and sequencing cDNA
- Study secondary structure and assembly characteristics in vitro
- Use synthetic peptides as model domains for in vitro assembly
- Compare SPs in other species

ACCOMPLISHMENTS

- structure of additional SPs obtained by cDNA cloning; expression of genes studied
- among all SPs studied, only one epitope is conserved among three species
- developed an assay for disassembly/reassembly of SPs and purifed spIs
- higher order structure of repeated domains obtained from model synthetic peptides

SIGNIFICANCE

- additional members of SP family chracterized
- conserved doamins suggest potential sites for protein interaction identified
- spIs are novel class of structual proteins with alternating α-helices and poly(Gly)II helices
- neither C nor SR peptides form supermolecular aggregates; thus assembly in vitro must require at least one [C+SR] core repeat